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FILE 'EMBASE, MEDLINE, BIOSIS, CAPLUS' ENTERED AT 08:49:08 ON 24 AUG 2001

L2 0 S L1 AND (DESOXYHEXOSE (S) REDUCTASE)
L3 18 S DESOXYHEXOSE
L4 212491 S REDUCTASE
L5 0 S L3 (S)L4
L6 0 S L3(W)L4
L7 16 DUP REM L3 (2 DUPLICATES REMOVED)
L8 19 S L1 AND ERYBII
L9 10 DUP REM L8 (9 DUPLICATES REMOVED)

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L9 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:546084 CAPLUS

DOCUMENT NUMBER: 133:147455

TITLE: Genes for enzymes of biosynthesis and transfer of 6-deoxy hexoses of Saccharopolyspora and Streptomyces and the development of novel macrolide antibiotics
INVENTOR(S): Fromentin, Claude; Michel, Jean Marc; Raynal, Marie Cecile; Salah, Bey Khadidja; Cortes, Jesus; Gaisser, Sabine; Leadlay, Peter; Mendez, Carmen; Salas, Jose

A.

PATENT ASSIGNEE(S): Hoechst Marion Roussel, Fr.

SOURCE: Fr. Demande, 211 pp.

CODEN: FRXXBL

DOCUMENT TYPE: Patent

LANGUAGE: French

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	---	-----	-----	-----
AB	FR 2786201	A1	20000526	FR 1999-3715	19990325
	Gene clusters assocd. with the biosynthesis and utilization of 6-deoxy hexoses in the biosynthesis of erythromycin are cloned and characterized for use in the manuf. of erythromycin and in the development of novel antibiotics. Sequences surrounding the ermE gene of S. erythraea were cloned and potential open reading frames identified using sequence homol. Inactivation of one of these genes (eryBII) by deletion resulted in the loss of the ability to synthesize erythromycin . The mutant accumulated erythronolide B and a no. of minor metabolites and detn. of their structures indicated that the gene encodes thymidine diphospho-4-keto-L-6-deoxyhexose 2,3-reductase. Similarly, the eryCIII gene was identified as encoding a desosaminyltransferase and eryCII encodes an isomerase.				

L9 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:546082 CAPLUS

DOCUMENT NUMBER: 133:161736

TITLE: Genes for enzymes of biosynthesis and transfer of 6-deoxy hexoses of Saccharopolyspora erythraea and Streptomyces antibioticus and their use in the development of novel macrolide antibiotics
INVENTOR(S): Fromentin, Claude; Michel, Jean Marc; Raynal, Marie Cecile; Salah, Bey Khadidja; Cortes, Jesus; Gaisser, Sabine; Leadlay, Peter; Mendez, Carmen; Salas, Jose

A.

PATENT ASSIGNEE(S): Hoechst Marion Roussel, Fr.

SOURCE: Fr. Demande, 210 pp.

CODEN: FRXXBL

DOCUMENT TYPE: Patent

LANGUAGE: French

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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	FR 2786189	A1	20000526	FR 1999-3716	19990325

AB Gene clusters assocd. with the biosynthesis and utilization of 6-deoxy hexoses in the biosynthesis of **erythromycin** are cloned and characterized for use in the manuf. of **erythromycin** and in the development of novel antibiotics. Sequences surrounding the ermE gene of *S. erythraea* were cloned and potential open reading frames identified using sequence homol. Inactivation of one of these genes (**eryBII**) by deletion resulted in the loss of the ability to synthesize **erythromycin**. The mutant accumulated erythronolide B and a no. of minor metabolites and detn. of their structures indicated that the gene encodes thymidine diphospho-4-keto-L-6-deoxyhexose 2,3-reductase. Similarly, the eryCIII gene was identified as encoding a desosaminyltransferase and eryCII encodes an isomerase.

L9 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:96365 CAPLUS
DOCUMENT NUMBER: 130:164011
TITLE: Ery and ole antibiotic biosynthesis genes and
Saccharopolyspora ery mutants for preparation of
novel
secondary metabolites and Streptomyces ole mutants
for
preparation of oleandomycin precursors
INVENTOR(S): Fromentin, Claude; Michel, Jean-Marc; Raynal,
Marie-Cecile; Salah-Bey, Khadidja; Cortes, Jesus;
Gaisser, Sabine; Leadlay, Peter; Mendez, Carmen;
Salas, Jose A.
PATENT ASSIGNEE(S): Hoechst Marion Roussel, Fr.
SOURCE: PCT Int. Appl., 222 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9905283	A2	19990204	WO 1998-FR1593	19980721
WO 9905283	A3	19990527		
W: BR, CA, JP, MX, TR, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
FR 2766496	A1	19990129	FR 1997-9458	19970725
FR 2786200	A1	20000526	FR 1998-7411	19980612
EP 1032679	A2	20000906	EP 1998-940290	19980721
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
JP 2001511349	T2	20010814	JP 2000-504257	19980721
PRIORITY APPLN. INFO.:			FR 1997-9458	A 19970725
			FR 1998-7411	A 19980612
			WO 1998-FR1593	W 19980721

AB Disclosed are the eryCII-eryCVI, **eryBII**, and ery BIV-eryBVII genes of *Saccharopolyspora erythraea* and the oleP1, oleG1, oleG2, oleM and
oleY genes of *Streptomyces antibioticus*. Addnl., *S. erythraea* ery deletion mutants and *S. antibioticus* ole deletion mutants may be used to prep. altered antibiotics or antibiotic precursors. A no. of ery and ole deletion mutants were prepd. The secondary metabolites produced by these mutant strains were detd.

L9 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:750895 CAPLUS
DOCUMENT NUMBER: 132:89154
TITLE: Transcriptional organization of the
erythromycin biosynthetic gene cluster of
Saccharopolyspora erythraea
AUTHOR(S): Reeves, Andrew R.; English, R. Samuel; Lampel, J. S.;

CORPORATE SOURCE: Post, David A.; Boom, Thomas J. Vanden
Fermentation Microbiology Research and Development,
Abbott Laboratories, North Chicago, IL, 60064-4000,
USA

SOURCE: J. Bacteriol. (1999), 181(22), 7098-7106
CODEN: JOBAAY; ISSN: 0021-9193

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The transcriptional organization of the **erythromycin**
biosynthetic gene (ery) cluster of *Saccharopolyspora erythraea* has been
examd. by a variety of methods, including S1 nuclease protection assays,
Northern blotting, Western blotting, and bioconversion anal. of
erythromycin intermediates. The anal. was facilitated by the
construction of novel mutants contg. a *S. erythraea* transcriptional
terminator within the eryAI, eryAIII, eryBIII, eryBIV, eryBV, eryBVI,
eryCIV, and eryCVI genes and addnl. by an eryAI -10 promoter mutant. All
mutant strains demonstrated polar effects on the transcription of
downstream ery biosynthetic genes. The results demonstrate that the ery
gene cluster contains four major polycistronic transcriptional units, the
largest one extending approx. 35 kb from eryAI to eryG. Two overlapping
polycistronic transcripts extending from eryBIV to eryBVII were
identified. In addn., seven ery cluster promoter transcription start
sites, one each beginning at eryAI, eryBI, eryBIII, eryBVI, and eryK and
two beginning at eryBIV, were detd.

REFERENCE COUNT: 41

REFERENCE(S): (2) Bailey, C; J Gen Microbiol 1986, V132, P2071
CAPLUS
(3) Bibb, M; Mol Microbiol 1994, V14, P533 CAPLUS
(4) Caballero, J; Mol Gen Genet 1991, V230, P401
CAPLUS
(5) Caffrey, P; FEBS Lett 1992, V304, P225 CAPLUS
(6) Church, G; Proc Natl Acad Sci USA 1984, V81,

P1991

CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 5 OF 10 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1

ACCESSION NUMBER: 1998121677 EMBASE

TITLE: Targeted gene inactivation for the elucidation of
deoxysugar biosynthesis in the **erythromycin**
producer *Saccharopolyspora erythraea*.

AUTHOR: Salah-Bey K.; Doumith M.; Michel J.-M.; Haydock S.; Cortes
J.; Leadlay P.F.; Raynal M.-C.

CORPORATE SOURCE: M.-C. Raynal, Infectious Disease Group, Hoechst Marion
Roussel, 102 Route de Noisy, 93235 Romainville Cedex,
France. marie-cecile.raynal@hmrag.com

SOURCE: Molecular and General Genetics, (1998) 257/5 (542-553).
Refs: 31
ISSN: 0026-8925 CODEN: MGGEAE

COUNTRY: Germany

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
039 Pharmacy

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The production of **erythromycin** A by *Saccharopolyspora erythraea*
requires the synthesis of dTDP-D-desosamine and dTDP-L-mycarose, which
serve as substrates for the transfer of the two sugar residues onto the
macrolactone ring. The enzymatic activities involved in this process are
largely encoded within the ery gene cluster, by two sets of genes
flanking
the eryA locus that encodes the polyketide synthase. We report here the

nucleotide sequence of three such ORFs located immediately downstream of eryA, ORFs 7, 8 and 9. Chromosomal mutants carrying a deletion either in ORF7 or in one of the previously sequenced ORFs 13 and 14 have been constructed and shown to accumulate erythronolide B, as expected for eryB mutants. Similarly, chromosomal mutants carrying a deletion in either ORF8, ORF9, or one of the previously sequenced ORFs 17 and 18 have been constructed and shown to accumulate 3- α -mycarosyl erythronolide B, as expected for eryC mutants. The ORF13 (eryBIV), ORF17 (eryCIV) and ORF7 (eryBII) mutants also synthesised small amounts of macrolide shunt metabolites, as shown by mass spectrometry. These results considerably strengthen previous tentative proposals for the pathways for the biosynthesis of dTDP-D-desosamine and dTDP-L-mycarose in *Sac. erythraea* and reveal that at least some of these enzymes can accommodate alternative substrates.

L9 ANSWER 6 OF 10 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1998:302096 BIOSIS

DOCUMENT NUMBER: PREV199800302096

TITLE: Analysis of eryBI, **eryBII** and eryBVII from the **erythromycin** biosynthetic gene cluster in *Saccharopolyspora erythraea*.

AUTHOR(S): Gaisser, S.; Bohm, G. A.; Doumith, M.; Raynal, M.-C.; Dhillon, N.; Cortes, J.; Leadlay, P. F. (1)

CORPORATE SOURCE: (1) Univ. Cambridge, Dep. Biochem., 80 Tennis Court Rd., Cambridge CB2 1GA UK

SOURCE: Molecular & General Genetics, (April, 1998) Vol. 258, No. 1-2, pp. 78-88.
ISSN: 0026-8925.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The gene cluster (ery) governing the biosynthesis of the macrolide antibiotic **erythromycin** A by *Saccharopolyspora erythraea* contains, in addition to the eryA genes encoding the polyketide synthase, two regions containing genes for later steps in the pathway. The region

5'

of eryA that lies between the known genes ermE (encoding the **erythromycin** resistance methyltransferase) and eryBIII (encoding a putative S-adenosylmethionine-dependent methyltransferase), and that contains the gene eryBI (orf2), has now been sequenced. The inferred product of the eryBI gene shows striking sequence similarity to authentic beta-glucosidases. Specific mutants were created in eryBI, and the resulting strains were found to synthesise **erythromycin** A, showing that this gene, despite its position in the biosynthetic gene cluster, is not essential for **erythromycin** biosynthesis. A mutant in eryBIII and a double mutant in eryBI and eryBIII were obtained and the analysis of novel **erythromycins** produced by these strains confirmed the proposed function of EryBIII as a C-methyltransferase. Also, a chromosomal mutant was constructed for the previously sequenced ORF19 and shown to accumulate erythronolide B, as expected for an eryB mutant and consistent with its proposed role as an epimerase in dTDP-mycarose biosynthesis.

L9 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:506684 CAPLUS

DOCUMENT NUMBER: 127:145934

TITLE: Cloning of genes eryB and eryC of *Saccharopolyspora erythraea* and their use for production of polyketides of modified glycosylation level

INVENTOR(S): Summers, Richard G., Jr; Katz, Leonard; Donadio, Stefano; Staver, Michael J.

PATENT ASSIGNEE(S): Abbott Laboratories, USA

SOURCE: PCT Int. Appl., 85 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9723630	A2	19970703	WO 1996-US20238	19961223
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5998194	A	19991207	US 1995-576626	19951221
EP 874548	A2	19981104	EP 1996-944476	19961223
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
JP 2000502899	T2	20000314	JP 1997-523805	19961223
PRIORITY APPLN. INFO.:			US 1995-576626	19951221
			WO 1996-US20238	19961223

AB A groups of genes encoding the enzymes involved in the biosynthesis of polyketide-assocd. sugars are isolated from *Saccharopolyspora erythraea*. Genes *eryB* and *eryC* assocd. with the biosynthesis of L-mycarose and D-desosamine, resp. By manipulation of the genes, a polyketide with novel glycosylation level can be produced. Prodn. of 4"-deoxy-4"-oxo-**erythromycin** A and other glycosylated polyketides in transgenic *Saccharopolyspora erythraea* was demonstrated.

L9 ANSWER 8 OF 10 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 2
 ACCESSION NUMBER: 97338861 EMBASE
 DOCUMENT NUMBER: 1997338861
 TITLE: Sequencing and mutagenesis of genes from the

erythromycin biosynthetic gene cluster of
Saccharopolyspora erythraea that are involved in

L-mycarose

and D-desosamine production.

AUTHOR: Summers R.G.; Donadio S.; Staver M.J.; Wendt-Pienkowski E.;

Hutchinson C.R.; Katz L.

CORPORATE SOURCE: L. Katz, Antibacterial Discovery Research Div, Abbott Laboratories, D-47P AP9A, 100 Abbott Park Road, Abbott Park, IL 60064, United States. leonard.katz@.abbott.com

SOURCE: Microbiology, (1997) 143/10 (3251-3262).

Refs: 59

ISSN: 1350-0872 CODEN: MROBEO

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The nucleotide sequence on both sides of the *eryA* polyketide synthase genes of the **erythromycin**-producing bacterium *Saccharopolyspora erythraea* reveals the presence of ten genes that are involved in L-mycarose (*eryB*) and D-desosamine (*eryC*) biosynthesis or attachment. Mutant strains carrying targeted lesions in eight of these genes indicate that three (*eryBIV*, *eryBV* and *eryBVI*) act in L-mycarose biosynthesis or attachment, while the other five (*eryCII*, *eryCIII*, *eryCIV*, *eryCV* and *eryCVI*) are devoted to D-desosamine biosynthesis or attachment. The remaining two genes (**eryBII** and *eryBVII*) appear to function in L-mycarose biosynthesis based on computer analysis and earlier genetic data. Three of these genes, **eryBII**, *eryCIII* and *eryCII*, lie between the *eryAIII* and *eryG* genes on one side of the polyketide synthase genes, while the remaining seven, *eryBIV*, *eryBV*, *eryCVI*, *eryBVI*, *eryCIV*, *eryCV* and *eryBVII* lie upstream of the *eryAI* gene on the other side of the gene cluster. The deduced products of these genes show similarities to: aldohexose 4-ketoreductases (*eryBIV*), aldoketo reductases (**eryBII**), aldohexose 5-epimerases (*eryBVII*), the *dnmT* gene of the daunomycin biosynthetic pathway of *Streptomyces peucetius* (*eryBVI*), glycosyltransferases (*eryBV* and *eryCIII*), the *AscC* 3,4-dehydratase from the ascarylose biosynthetic pathway of *Yersinia pseudotuberculosis*

(eryCIV), and mammalian N-methyltransferases (eryCVI). The eryCII gene resembles a cytochrome P450, but lacks the conserved cysteine residue responsible for coordination of the haem iron, while the eryCV gene displays no meaningful similarity to other known sequences. From the predicted function of these and other known eryB and eryC genes, pathways for the biosynthesis of L-mycarose and D-desosamine have been deduced.

L9 ANSWER 9 OF 10 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 3
ACCESSION NUMBER: 90141730 EMBASE
DOCUMENT NUMBER: 1990141730
TITLE: Organization of a cluster of **erythromycin** genes
in *Saccharopolyspora erythraea*.
AUTHOR: Weber J.M.; Leung J.O.; Maine G.T.; Potenz R.H.B.; Paulus
T.J.; DeWitt J.P.
CORPORATE SOURCE: BioProcess Development, D451-R5, Abbott Laboratories, North
Chicago, IL 60064, United States
SOURCE: Journal of Bacteriology, (1990) 172/5 (2372-2383).
ISSN: 0021-9193 CODEN: JOBAAY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We used a series of gene disruptions and gene replacements to
mutagenically characterize 30 kilobases of DNA in the **erythromycin**
resistance gene (ermE) region of the *Saccharopolyspora erythraea*
chromosome. Five previously undiscovered loci involved in the
biosynthesis
of **erythromycin** were found, eryBI, **eryBII**, eryCI,
eryCII, and eryH; and three known loci, eryAI, eryG, and ermE, were
further characterized. The new Ery phenotype, EryH, was marked by (i) the
accumulation of the intermediate 6-deoxyerythronolide B (DEB), suggesting
a defect in the operation of the C-6 hydroxylase system, and (ii) a block
in the synthesis or addition reactions for the first sugar group.

Analyses
of ermE mutants indicated that ermE is the only gene required for
resistance to **erythromycin**, and that it is not required for
production of the intermediate erythronolide B (EB) or for conversion of
the intermediate 3-.alpha.-mycarosyl erythronolide B (MEB) to
erythromycin. Mutations in the eryB and eryC loci were similar to
previously reported chemically induced eryB and eryC mutations blocking
synthesis or attachment of the two **erythromycin** sugar groups.
Insertion mutations in eryAI, the macrolactone synthetase, defined the
largest (at least 9-kilobase) transcription unit of the cluster. These
mutants help to define the physical organization of the
erythromycin gene cluster, and the eryH mutants provide a source
for the production of the intermediate DEB.

L9 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:76305 CAPLUS
DOCUMENT NUMBER: 114:76305
TITLE: Cloning of cluster of **erythromycin**
biosynthesis genes from *Streptomyces erythraeus*
AUTHOR(S): Ukhabotina, L. S.; Danilenko, V. N.; Navashin, S. M.
CORPORATE SOURCE: All-Union Res. Inst. Antibiot., Moscow, USSR
SOURCE: Antibiot. Khimioter. (1990), 35(12), 3-7
CODEN: ANKHEW; ISSN: 0235-2990
DOCUMENT TYPE: Journal
LANGUAGE: Russian

AB The **erythromycin** resistance gene ermE along with
erythromycin formation genes eryBI, **eryBII**, eryCI,
eryCII, eryD, and eryH of the same gene cluster of *S. erythraea* were
cloned in plasmid pUC18 and phage .lambda.EMBL3. A cloned DNA fragment
of
.apprx.20 kb in .lambda.EMBL3 was colinear with genomic DNA of *S.*

erythraea. Subcloning in plasmid pUC18 resulted in the isolation of plasmids harboring BamHI restricted DNA from the *S. erythraea* chromosomal region that contained *ermE*. The cloned genes for **erythromycin** formation and *ermE* may be used for the identification and subsequent isolation of genes for polyketide antibiotic biosynthesis.